

Effects of Rootstock on the Composition of Bergamot (*Citrus bergamia* Risso et Poiteau) Essential Oil

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This paper reports the composition of bergamot oils obtained from plants grafted on the following rootstocks: sour orange, Carrizo citrange, trifoliate orange, Alemow, Volkamerian lemon, and Troyer citrange. The aim of this study is to evaluate the possibility of using rootstocks other than sour orange, checking their effect on the composition of the essential oil. Results are reported for analysis of 203 bergamot oils during the years 1997–1998, 1998–1999, and 1999–2000. The oils were analyzed by HRGC and HRGC/MS; 78 components were identified, and the results were in agreement with those reported in the literature for the Calabrian bergamot oils obtained from industry. Because of the quality of their essential oils, Alemow and Volkamerian lemon can be considered as substitutes for sour orange rootstocks.

KEYWORDS: Bergamot (*Citrus bergamia* Risso et Poiteau); essential oil composition; rootstock; HRGC; HRGC/MS

INTRODUCTION

Bergamot (*Citrus bergamia* Risso et Poiteau) is mainly cultivated for its essential oil—an important raw, widely used substance that has great commercial value. It is necessary in the international perfumery industry as it has not only the function of fixing the aromatic bouquets of perfumes, but also that of blending all the other essences contained in them, exalting notes of freshness and fragrance. Bergamot essential oil is used in the pharmaceutical industry, mainly in dentistry, ophthalmology, gynecology, and dermatology. In fact, it is included in the official pharmacopoeias of various countries, Italy included. Bergamot essential oil is also used in the food and confectionery industries as a flavoring for liqueurs, teas, toffees, candies, ice creams, and soft drinks (1, 2).

Ninety-five percent of worldwide bergamot cultivation occurs in the Ionic area of Calabria, Italy; alluvial and clayey-limic soils, where pH is less than 6.5–7.5, are particularly suitable for cultivating bergamot. Bergamot is reproduced only through grafting. Years ago it was grafted on two-year-old plants of limetta or of sour orange. Today, grafting of bergamot on limetta has been abandoned because of the lack of resistance of the

tree to diseases. Among different techniques aiming toward an improvement of the quality and quantity of bergamot cultivation is grafting of the three main bergamot cultivars (castagnaro, femminello, and fantastico) onto different rootstocks. The rootstock is very important for the plant, and it must be chosen with great attention because it cannot be changed or modified if not satisfactory, unlike in common cultivation. According to the kind of rootstock used, different morphological and biological characteristics—for example, plant growth and fruit production—are obtained (3, 6).

The influence of the rootstock on the quality of bergamot oil has not been studied, while there are various papers on the composition of industrial bergamot oil produced in Calabria (7, 11).

The aim of this study is to evaluate the possibility of using different rootstocks from sour orange, checking their effect on the composition of the essential oil.

Here are report results regarding bergamot essential oil from Castagnaro plants grafted on different rootstocks during the years 1997–1998, 1998–1999, and 1999–2000.

MATERIALS AND METHODS

The research was carried out on 203 samples of bergamot (*Citrus bergamia* Risso et Poiteau). The fruits were picked during the years 1997–1998, 1998–1999, and 1999–2000 in Istituto Sperimentale per l'Agricoltura di Reggio Calabria, Italy. The plants were planted in 1988 at one-sixth of 5 × 5 m², respecting a layout in block, and sour orange (*Citrus aurantium* L.), Carrizo citrange (*Citrus sinensis* (L.)

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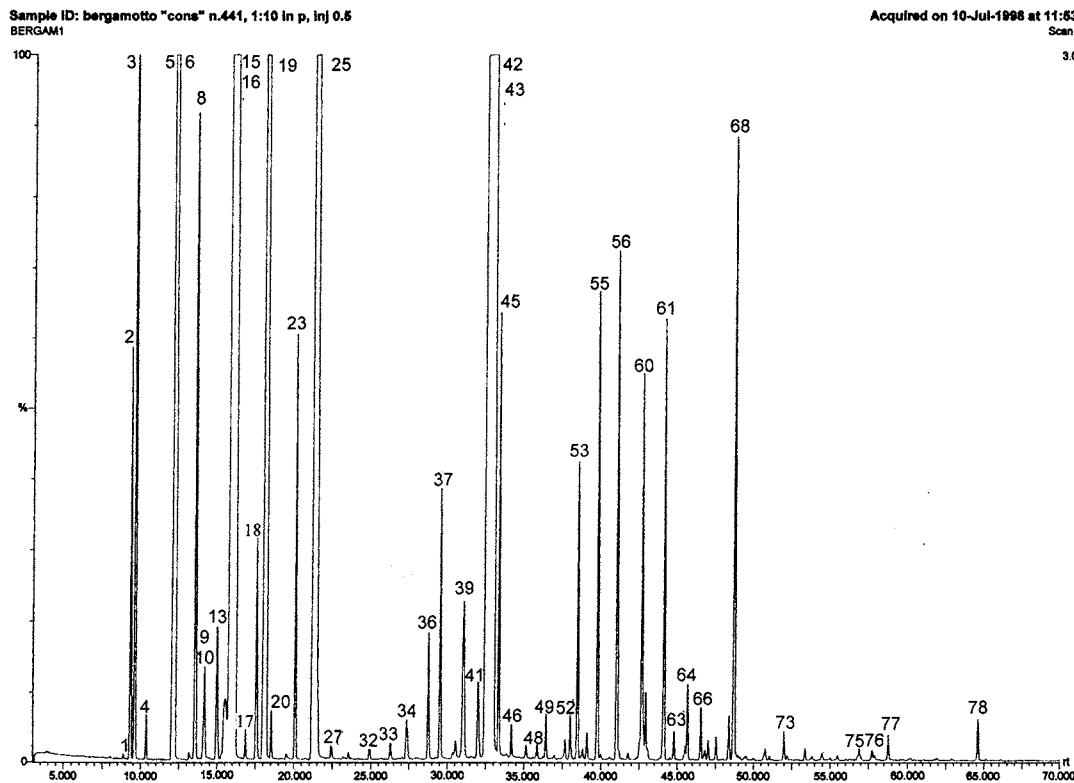


Figure 1. HRGC/MS chromatogram of bergamot essential oil.

Table 1. Samples Analyzed of Bergamot Essential Oils

rootstock	production year			all
	97–98	98–99	99–00	
sour orange	13	11	14	38
Carrizo citrange	12	10	10	32
Alemow	12	11	14	37
trifoliolate orange	9	10	11	30
Volkamerian lemon	13	10	9	32
Troyer citrange	9	12	13	34
all	68	64	71	203

Osbeck x *Poncirus trifoliata* (L.) Raf.), trifoliolate orange (*Poncirus trifoliata* (L.) Raf.), Alemow (*Citrus macrophylla* Wester), Volkamerian lemon (*Citrus Volkameriana* Ten. and Pasq.), and Troyer citrange (*Citrus sinensis* (L.) Osbeck cv. "Washington navel" x *Poncirus trifoliata* (L.) Raf.) were used as rootstocks. Rootstocks are grafted with Castagnaro cultivar. Information on the samples analyzed is given in Table 1.

Each essential oil was prepared by selecting and peeling five ripe, regular, sound fruits from 12 fruits batches, four fruits for each bergamot plant collected following the cardinal point. Extraction of the essential oil was carried out in the laboratory by applying manual pressure on the rind so as to cause the breaking of the utricles and the release of the oil itself, which was collected on a watch glass, transferred to a test tube, centrifuged, and analyzed. Fruit and peel weight, peel thickness, and essential oil yield are reported in Table 2.

The volatile fraction of each oil was studied by HRGC and HRGC/MS.

HRGC/MS Analysis. For the identification of volatile components, each sample was analyzed by using a Shimadzu QP 5000 equipped with Adams's library (12) on two different columns: (1) Fused silica capillary column, 30 m x 0.25 mm i.d., coated with Mega 5MS, 0.25 μ m film thickness (Mega, Legnano (MI) Italy); column temperature, 40 °C (2 min) to 240 °C at 3.0 °C/min; carrier gas He, 90 kPa; linear velocity, 42.7 cm/s at 40 °C. (2) Fused silica capillary column, 30 m x 0.25 mm, coated with Megawax, 0.25 μ m film thickness (Mega,

Legnano (MI) Italy); column temperature, 40 °C (6 min) to 220 °C (10 min) at 2.0 °C/min; carrier gas He, 90 kPa; linear velocity, 42.8 cm/s at 40 °C. For both columns: injector temperature, 250 °C; injection mode, split; split ratio, 1:100; volume injected, 1 μ L of a solution 1/20 in pentane of the oil. MS scan conditions: interface temperature, 250 °C; source temperature, 200 °C; E energy, 70 eV; mass scan range, 41–300 amu.

Linear retention indices of the sample components were determined on the basis of homologue *n*-alkane hydrocarbons analyzed under the same GC conditions. The compound identification was confirmed by standard injection and comparison of compounds' mass spectra to published spectra and retention indices to published index data (13).

HRGC Analysis. For quantitative results of the volatile fraction, each sample was analyzed by HRGC on a Fisons Mega Series 5160 gas chromatograph equipped with a Shimadzu C-R3A data processor; fused silica capillary column, 30 m x 0.32 mm i.d., coated with SE-52, 0.40–0.45 μ m film thickness (Mega, Legnano (MI), Italy); column temperature, 45 °C (6 min) to 200 °C at 3 °C/min; injection mode, split; split ratio, 1:100; detector, FID; injector and detector temperature, 250 °C; carrier gas, He 95 kPa; injected volume, 1 μ L of neat oil. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to be equal to 1.

RESULTS AND DISCUSSION

All data regarding the plant growth—data related to fruit included—have not shown remarkable differences for what concerns the rootstocks. Nonetheless, a more fruitful production has been obtained in plants grafted on Alemow and on Volkamerian lemon, and the least fruitful production has been obtained in plants grafted on trifoliolate orange. The essential oil yield has not been influenced by rootstocks (Table 2). In evaluating the quality of bergamot essential oil from different rootstocks, the volatile fraction has been analyzed. The HRGC/MS chromatogram obtained on Mega 5MS is reported in Figure 1. The components identified in the oils are reported in Table

Table 2. Average Values of Fruit and Peel Weights, Peel Thickness, and Essential Oil Yield for the Samples Analyzed (Each Sample: Five Fruits)

	fruit weight (kg)		peel weight (g)		peel thickness (mm)		essential oil (g)	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
Alemow (38) ^a	1.25	0.133	268.63	8.106	4.56	0.496	5.20	1.669
Carrizo citrange (32)	1.20	0.134	278.67	4.241	4.65	0.419	5.89	1.229
sour orange (37)	1.13	0.181	261.96	2.924	4.62	0.724	5.09	1.069
trifoliate orange (30)	1.10	0.198	232.42	6.129	4.42	0.384	5.14	1.201
Troyer citrange (34)	1.11	0.226	240.40	7.571	4.32	0.435	5.04	1.305
Volkamerian lemon (32)	1.31	0.261	328.00	7.271	4.77	0.614	6.31	2.106

^a In parentheses is the number of samples analyzed.

Table 3. Retention Indices on Mega 5MS and Megawax for the Components Identified in Bergamot Oils

component	Mega 5MS	Megawax	component	Mega 5MS	Megawax
tricyclene	913	nd	<i>trans</i> -sabinene hydrate acetate	1246	1501
α -thujene	920	1012	linalyl acetate	1251	1542
α -pinene	925	1008	geraniol	1251	1783
camphene	936	1043	(<i>E</i>)-dec-2-en-1-al	1254	nd
sabinene	964	1105	geraniol + perillaldehyde	1262	1697
β -pinene	964	1085	bornyl acetate	1275	nd
6-methyl-5-hepten-2-one	981	nd	indol	1290	nd
myrcene	987	1152	undecanal	1300	1580
octanal	997	1270	nonyl acetate	1308	nd
α -phellandrene	997	1146	methyl geranate	1316	nd
hexyl acetate	1000	nd	linalyl propionate	1333	nd
δ -3-carene	1002	1128	δ -elemene	1328	1446
α -terpinene	1009	1160	α -terpinyl acetate	1340	1663
<i>p</i> -cymene	1016	nd	citronellyl acetate	1349	nd
limonene + β -phellandrene	1021	1181	neryl acetate	1360	1705
1,8-cineole	1021	nd	geranyl acetate	1379	1738
(<i>Z</i>)- β -ocimene	1036	nd	dodecanal	1402	nd
(<i>E</i>)- β -ocimene	1045	1239	decyl acetate	1404	1662
γ -terpinene	1051	1225	<i>cis</i> - α -bergamottene	1405	nd
<i>cis</i> -sabinene hydrate	1058	1447	β -cariofillene	1404	1555
octanol	1071	1546	<i>trans</i> - α -bergamottene	1427	1560
<i>cis</i> -linalool oxide	1075	nd	α -umulene	1437	1624
terpinolene	1079	1260	(<i>Z</i>)- β -farnesene	1452	1649
<i>trans</i> -linalool oxide	1094	1537	<i>cis</i> - β -santalene	1449	nd
linalool	1094	nd	dodecanol	1460	nd
nonanal	1098	1372	germacrene D	1466	1664
heptyl acetate	1111	nd	bicyclogermacrene	1482	1688
<i>cis</i> -limonene oxide	1122	1403	(<i>Z</i>)- α -bisabolene	1495	nd
<i>trans</i> -limonene oxide	1127	1423	(<i>E</i>)- α -farnesene	1502	1730
isopulegol	1132	nd	β -bisabolene	1500	1701
camphor	1127	nd	<i>cis</i> - γ -bisabolene	1504	nd
citronellal	1146	1457	germacrene B	1539	1778
terpinen-4-ol	1165	1575	(<i>E</i>)-nerolidol	1556	nd
α -terpineol	1179	1670	tetradecanal	1605	nd
dodecane	1185	nd	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	1638	nd
decanal	1199	1476			
octyl acetate	1210	1461	campherenol	1654	nd
nerol + citronellol	1222	1830	α -bisabolol	1657	nd
neral	1231	1645	nootkatone	1780	2434
carvone	1229	1684			

3, together with the linear retention indices calculated on Mega 5MS and on Megawax columns.

The average composition and the standard deviation as classes of substances and as single components for the oils analyzed, grouped according to the rootstock, are reported in **Table 4**.

In each rootstock, the high standard deviations for many components are due to their variability during the productive seasons.

The compounds identified in the oil analyzed were the same, and the amount of almost all the compounds and classes of substances was included in the range reported for the industrial Calabrian bergamot oils (10, 11). Seventy-eight components were identified in each oil, which constitute more than 99.9% of the whole volatile fraction.

For all samples, the main compounds were limonene, linalyl acetate, linalool, γ -terpinene, sabinene, and β -pinene. Monoterpenes were the main class of substances. Moreover, the oils were characterized by a high amount of alcohols and esters, and about 99% of these classes were made up of linalool and linalyl acetate. There were only small amounts of carbonyl compounds and of sesquiterpene hydrocarbons. Linalool (light, lavender) and linalyl acetate (fruity, floral, lavender) marker the flavor notes of the bergamot oil; neral and geraniol (lemon) and neryl and geranyl acetate (fruity, floral, rose) are important, too. For these reasons, international marker evaluates the quality of a bergamot oil according to the amount of oxygenated compounds and, in particular, of linalool and linalyl acetate.

Table 4. (Continued)

	sour orange		Carrizo citrange		Alemow		trifoliolate orange		Volkamerian lemon		Troyer citrange	
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
75) 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol ^b	tr ^a		tr ^a		tr ^a		tr ^a		tr ^a		tr ^a	
76) campherenol ^b	tr ^a		tr ^a		tr ^a		tr ^a		tr ^a		tr ^a	
77) α -bisabolol	tr ^a		tr ^a		tr ^a		tr ^a		tr ^a		tr ^a	
78) nootkatone	0.1	0.01	tr ^a		tr ^a		tr ^a		tr ^a		tr ^a	
hydrocarbons	47.7	12.85	54.1	15.22	49.8	12.20	59.3	8.75	47.7	8.74	50.4	8.63
monoterpenes	46.7	12.65	53.1	14.97	49.0	12.01	58.2	8.51	46.8	8.64	49.3	8.54
sesquiterpenes	1.0	0.21	1.0	0.25	0.8	0.19	1.1	0.24	0.9	0.10	1.1	0.09
alcohols	13.9	4.72	11.2	3.93	11.9	3.54	8.3	2.36	15.0	4.31	13.7	4.38
esters	37.5	5.05	33.7	6.52	37.6	3.53	30.9	3.32	36.5	4.10	35.0	3.52
carbonyl compounds	0.7	0.10	0.5	0.08	0.5	0.11	0.5	0.12	0.6	0.14	0.6	0.17
oxygenated compounds	52.1	9.86	45.4	10.52	50.0	7.18	39.7	5.80	52.1	8.55	49.3	8.07
linalol/linalyl acetate	0.37		0.34		0.32		0.27		0.41		0.39	

^a tr = <0.01. ^b Tentative identification.

The enantiomeric distribution of some components, such as linalool and linalyl acetate, has been determined. The data were in accord with the results previously obtained for genuine cold-pressed bergamot oils, and no differences appeared according to the rootstock (14).

The average compositions of oils from each rootstock during the analyzed years appear similar. From 1997–1998 to 1999–2000, only a slight increase was observed in the average content of monoterpene hydrocarbons—in particular of limonene—and a slight decrease in the average content of linalool and linalyl acetate. This difference has been detected in oils from all rootstocks, and it is probably due to the different climatic conditions during those years.

As can be seen from Table 4, different quantitative compositions as single components and, obviously, as classes of substances were observed for the bergamot oils according to the rootstock. For example, the limonene content varies from \bar{X} = 34.2% (sour orange) to \bar{X} = 41.4% (trifoliolate orange), linalool from \bar{X} = 8.2% (trifoliolate orange) to \bar{X} = 14.8% (Volkamerian lemon), and linalyl acetate from \bar{X} = 29.9% (trifoliolate orange) to \bar{X} = 36.6% (sour orange).

Bergamot oils obtained using sour orange as rootstock (which is the traditional rootstock) have an average content of oxygenated compounds of 52.1% and a content of hydrocarbons of 47.7%. These oils have the highest average content of linalyl acetate, \bar{X} = 36.6%, and a good quantity of linalool, \bar{X} = 13.7%, with a proportion between these two of 0.37. The analyzed oils that are more similar to those found grafted on sour orange, especially in terms of linalool and linalyl acetate contents, are those found grafted on Alemow and Volkamerian lemon. The oils obtained using trifoliolate orange are the most different from sour orange and have the lowest content of linalool (\bar{X} = 8.2%), linalyl acetate (\bar{X} = 29.9%), and oxygenated compounds (\bar{X} = 39.7%)

In conclusion, it is possible to state that, because of their productiveness and the quality of their essential oils, Alemow and Volkamerian lemon can be considered as substitute rootstocks for sour orange.

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Received for review June 20, 2002. Revised manuscript received September 13, 2002. Accepted September 14, 2002.

JF0206872